ENZYME HYDROLYSIS OF OLD NEWSPAPER TO PRODUCE SIMPLE SUGAR FOR FERMENTATION PROCESS

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AFEX	Ammonia fiber explosion
DNS	3,5-dinitrosalicyclic acid
NP	Nonylphenol
OFAT	One-factor-at-a time
TW	Tween
UV-Vis	Ultraviolet-visible

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ABSTRAK

Penghasilan penapian gula dari bahan lignoselulosa telah dikaji oleh ramai penyelidik. Pada masa kini, banyaknya terhasil bahan buangan lignoselulosa disekeliling dunia termasuk juga di Malaysia. Surat khabar adalah sumber bahan lignoselulosa kerana surat khabar itu sendiri diperbuat daripada kayu lembut atau pulpa kayu yang mengandungi bahan lignoselulosik iaitu selulosa, separa-selulosa dan lignin. Selulosa adalah komponen terbesar pembahagiannya dengan 45 – 50 %. Selulosa juga boleh ditukarkan kepada gula mudah iaitu glukosa. Hidrolisis enzim lebih digalakkan untuk proses penukaran kerana lebih jimat dan kadar penukaran yang lebih banyak. Dalam kajian ini, enzim cellulase dari Novozyme telah digunakan kepada hidrolisis surat khabar untuk penukaran glukosa. Dengan mengunakan satu-faktor-pada- satu masa (OFAT), enam pembolehubah telah dikaji. Pembolehubahnya adalah nibah surfaktan, penambahan enzim, penampan pH, suhu pengeraman, kelajuan pergolakkan dan waktu pengeraman. Didalam ekperimen ini, penghasilan glukosa telah diukur mengunakan kaedah DNS. Keputusan yang dapat telah menunjukkan pada nisbah surfaktan 0.2:0.8 untuk TX-21:TWEEN-80. 1.0 ml enzim cellulase pada pH 6.0 apabila dimasukkan bersama 0.2 g surat khabar pada 50 ^{o}C dan pergolakan 200 rpm untuk 96 jam adalah sesuai untuk reaksi enzim. Pembolehubah-pembolehubah yang telah dapat memberikan penghasilan glukosa yang tertinggi iaitu pada 0.73 g/L.

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ABSTRACT

Production of fermentable sugar from lignocellulosic material was attempted by many researchers. Nowadays, this is because of plenty lignocellulosic biomass waste available around the world including Malaysia. Newspaper is a type of lignocellulosic sources because newspaper was made from softwood that contains cellulose, hemicelluloses and lignin. Cellulose component in newspaper is the major component with 45-50 %. The celluloses were able to be converted into simple sugar, which is glucose. Enzymatic hydrolysis is preferred for the conversion because less cost and high conversion. In this study, cellulase enzyme from Novozyme was used to hydrolyze newspaper for glucose production. By applying one-factor-at-time (OFAT), six parameters were being studied. They are surfactant ratio, enzyme loading, buffer pH, incubation temperature, agitation speed and incubation time. In all experiments, glucose production was monitored by DNS method. The result obtained show that at surfactant ratio 0.2:0.8 of TX-21: TWEEN-80, 1.0 ml of cellulase enzyme, at pH 6.0 when mixed with 0.2 g substrate at 50 ^{o}C and agitated at 200 rpm for 96 h is suitable for enzyme reaction. Those optimum parameters obtained gave the higher glucose production at 0.73 ± 0.02 g/L.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Lignocellulose biomass is the most abundant raw material waste on the earth. Abundant newspaper waste was generated every year from all over the world due to high production rate of newspapers. This has become one of the severe environmental issues.

Lignocellulosic biomass is favorable for its highly abundance and high energy potential. Lignocellulosic materials consist of three major component, they are cellulose, hemicelluloses and lignin. The bioconversion of lignocellulosic materials is now a subject of intensive research as a contribution to the development of large scale conversion

process beneficial to mankind (Kumakura, 1997). In newspaper, cellulose comprises the largest fraction of the biomass ranging from 30% to 50% (MacLellan, 2010) that can be degrading to smaller monomer of fermentable sugar such as glucose. In fermentation process, a carbon source is the most important feed to produce valuable product. In this case, glucose is widely use in fermentation process for such purpose.

Lignocellulosic materials are complex carbon source that need to go through pretreatment process. Only enzyme can fully degrade the newspaper to fermentable sugar, which is called enzymatic hydrolysis. Enzymatic hydrolysis is a process of degrading biomass sample using enzyme under suitable condition to produce fermentable sugar. In pretreatment of lignocellulosic material, enzymatic hydrolysis is preferred because of the higher conversion yield, less corrosive, less toxic condition as compared to others pretreatment method such as physical pretreatment, physico-chemical pretreatment, chemical pretreatment and biological pretreatment (Sun, 2002). The enzyme that can degrade cellulose to glucose is cellulase enzyme.

Cellulases are among the industrially important hydrolytic enzymes and are of great significance in present day biotechnology. Cellulose hydrolysis is accomplished with the aid of cellulase enzyme complex which is made up of three classes of enzymes namely exoglucanase, endoglucanase and β-glucosidase (Beguin, 1990). Cellulase enzyme is a group of enzyme that acting to break the polymer chain of cellulose to monomer chain of glucose (MacLellan, 2010). Because of too many factors need to considered, all the parameters during the process have to be examined in order to understand the importance and effect of the parameters for optimizing the production of glucose from newspaper.

1.2 Problem Statement

To obtain the higher fermentable glucose production from old newspaper using cellulase enzyme by enzymatic hydrolysis, recognizing the important parameters that affect enzymatic hydrolysis are very important. Furthermore, Novozyme Cellulase enzyme complex, is a newly developed commercial enzyme, there is no study has been done in its application on hydrolyzing newspaper to glucose production. Therefore, investigation on the parameters that affect enzymatic hydrolysis is necessary.

1.3 Research Objective

To study the effect of various operating parameters during enzymatic hydrolysis of newspapers to fermentable glucose by cellulose.

1.4 Scope Of Study

The scope of study was to determine the effect of all operating parameters that would influence enzymatic hydrolysis in producing high amount of glucose i.e. surfactant ratio, pH buffer, enzyme loading, agitation speed, incubation temperature and incubation time.

1.5 Significant Of Proposed Study

Current world trend has moved towards paperless concept. However, in certain countries and certain tasks, such as legal document, newspaper and magazine, still need to use paper especially in Malaysia. Popularity of using paper has generated lots of paper waste per annum. Then, these papers were collected and recycled to produce recycled paper, but after several cycle, the paper quality decrease and this give disadvantaged to the biomass content because the conversion will be lower. Instead of generating tons of waste every year, this biomass can be used to convert to more valuable product such as glucose or as feedstock to generate renewable energy that can replace current petroleum based liquids fuels or others bioproducts.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview

Production of newspaper per annum was abundance in Malaysia because newspaper was produced everyday. Besides that, the used newspaper has been recycled as recycle paper, but recycled paper is low in quality and if recycle for several time, it will become useless product. Therefore, in order to generate less solid waste, newspaper should be transformed to some other valuable products. Through this study, the old newspaper was used to convert the cellulose component in it into the sugar, where this sugar was able to produce another more valuable product such as biofuel. Newspaper is

made from softwood that contained lignocellulosic materials, where the major component is cellulose. Enzymatic hydrolysis of newspaper by cellulase enzyme is preferred because of its ability to convert the cellulose into high quantity of fermentable sugar (Chu and Feng, 2012), which is a more efficient pretreatment method compared to others. To improve and obtain the optimum value for production of glucose, all factors that affecting the process will be examined starting from initial pretreatment until enzymatic hydrolysis of newspaper.

2.2 Introductions of Lignocellulosic Materials

Sun and Cheng (2002) stated that cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. According to Rao (2009), it is composed of about 30-50 % cellulose, 20-35 % of hemicelluloses and 10-15 % lignin. This linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bonds. Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils (Zhang and Lynd, 2004). Hemicelluloses and lignin, on the other hand, are found cover the microfibrils. Fermentable D-glucose can be produced from cellulose through the action of either acid or enzymes breaking the β-(1,4)-glycosidic linkages (Gusakov *et. al.*, 2006).

Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form. The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses, hexoses, and uronic acids. The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by β-(1, 4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds. Also, hemicelluloses can have some degree of acetylation. In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolyzable. These polymers do not aggregate, even when they cocrystallize with cellulose chains.

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack. Three phenyl propionic alcohols exist as monomers of lignin: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol). Alkyl−aryl, alkyl−alkyl, and aryl−aryl ether bonds link these phenolic monomers together. In general, herbaceous plants such as grasses have the lowest contents of lignin, whereas softwoods have the highest lignin contents (Kumar *et. al.*, 2009).

2.3 Pretreatment of Substrate by Non-Ionic Surfactant

To improve the accessibility of the substrate, which is old newspaper, it is required to pretreat it before undergo to sugar production. As stated by Kim *et. al.* (2007), cellulose is the major component of newspaper that is able to convert into fermentable sugars by enzymatic hydrolysis. Enhancement of cellulose hydrolysis by adding surfactants to the hydrolysis mixture has been reported by (Chandra *et. al.*, 2008). Eriksson *et. al.* (2002) was compared amorphous cellulose with different types of crystalline celluloses (Avicel, tissue paper and reclaimed paper). They showed that the higher the crystallinity of the substrate, the more positive was the effect of the added surfactant.

However, inks and certain additives used in paper production can hinder enzyme access to the substrate. Thus, an effective pretreatment is an essential step to increase the enzymatic digestibility of waste paper and reducing quantity of enzyme. According to Kumar *et. al.* (2009) and also Sun and Cheng (2002), the composition of lignocellulosic material of newspaper is cellulose 40-55 %, hemicelluloses 25-40 % and lignin 18-30 %. Kim and Feng (2007) believe that surfactants can help to remove the ink and other components that physically interfere with enzymatic hydrolysis.

Studies of pretreatments that were based on surfactants alone are very limited. Furthermore, the addition of a surfactant to the enzymatic hydrolysis stage of lignocelluloses increases the conversion of cellulose to glucose. When newspaper was pretreated to increase its enzymatic digestibility, the pretreatments that based on a surfactant alone showed higher digestibility than the pretreatments that based on Tween-

series surfactants, ammonia, and/or hydrogen peroxide. According to Hwang *et. al.* (2008), TWEEN-80 has been proposed because TWEEN 80 able to increased microbial growth, cellulolytic enzyme activity and enzyme binding on substrate. Therefore, TWEEN-80 may improve fiber digestion and made enzymatic degradation of cellulose easier. Moreover, non-ionic surfactant decreases the absorption of enzyme to substrate, which may aid to maintain enzymatic reaction. According to Kim *et. al.* (2007) in their preliminary experiments, NP-20 showed slightly better performance than NP-5 and NP-10, and therefore, NP-20 was selected for further experimentation together with TW-80 on pretreatment performance. The digestibility of the NP-20- pretreated substrate was approximately 10 % higher than that of TW-80-pretreated substrate. Significant surfactant effect with addition NP-20 and TWEEN-80 were give high digestibility α-cellulose of newspaper compares to small surfactant,

In addition, the surfactant TX-21 that has similar characteristic such as hydroxyl value at 44.54 mgKOH/g, the moisture content at 1.0 % with pH 5.0 – 7.0 with the NP-20 that can give similar behave if mix with TWEEN-80. Therefore, the reason of combination TWEEN-80 with TX-21 is to complete the process where TWEEN-80 was de-inking the newspaper and TX-21 remove all the impurities including surfactants during substrate washing with deionized water (Kim *et. al.*, 2007).

2.4 Pretreatment of Lignocellulosic Materials

Pretreatment of lignocellulosic materials can convert cellulose to fermentable glucose. Glucose acts as carbon source that is an important nutrient for microorganism to produce valuable product such as biofuels. The aim of pretreatment process is to remove lignin and hemicelluloses, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment must have the following requirement. First is proving the formation of sugar or the ability to subsequently form sugar by hydrolysis. Second is to avoid the degradation or loss of carbohydrate. Third is avoid the formation of byproducts that are inhibitory to the subsequently hydrolysis and fermentation process and last is be cost-effective. Pretreatment methods can be divided into different categories which are physical (milling and grinding), physicochemical (steam pretreatment, hydrothermolysis and wet oxidation), chemical (alkali, dilute acid, oxidizing agent and organic solvent), biological or electrical (Kumar *et. al.*, 2009).

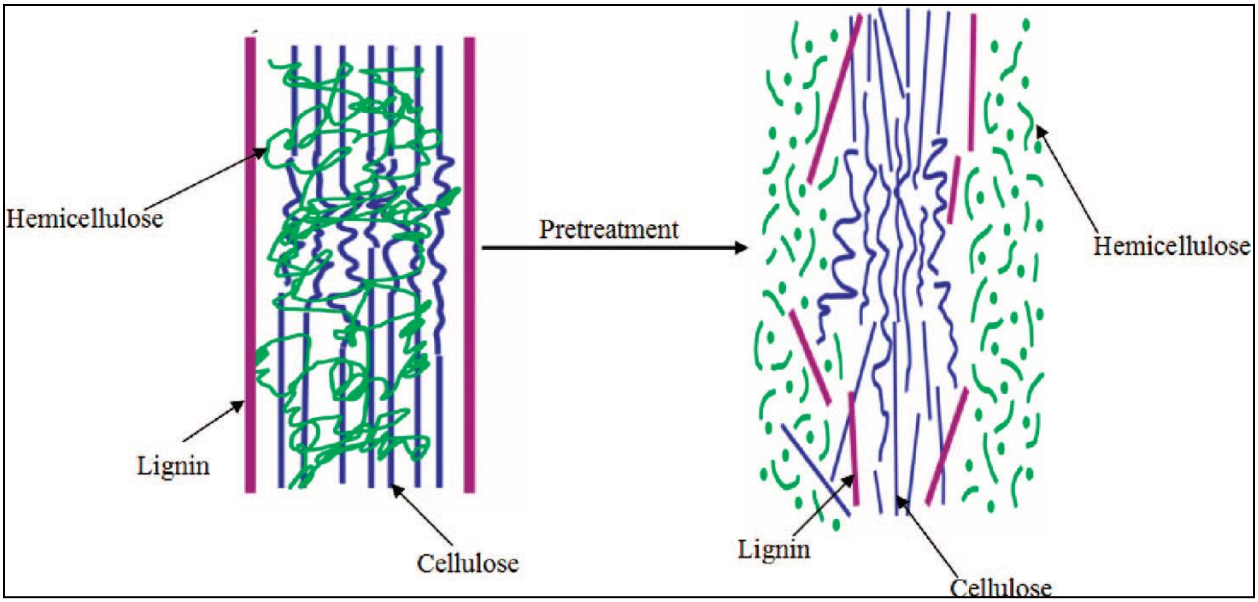

Figure 2.1 An illustration of pretreatment lignocellulosic materials by surfactant. (Source: Kumar *et. al*, 2009)

2.4.1 Physical Pretreatment

2.4.1.1 Mechanical Comminution

Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling. The power

requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass. (Sun and Cheng, 2002; Kumar *et. al.*, 2009)

2.4.1.2 Pyrolysis

Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 ^{o}C, cellulose rapidly decomposes to produce gaseous products and residual char. The decomposition is much slower and less volatile products are formed at lower temperatures. Mild acid hydrolysis (1 N H_2SO_4, 97 ^{o}C, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80–85 % conversion of cellulose to reducing sugars with more than 50 % glucose. The process can be enhanced with the presence of oxygen. When zinc chloride or sodium carbonate is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature (Sun and Cheng, 2002; Kumar *et. al.*, 2009)

2.4.2 Physico-Chemical Pretreatment

2.4.2.1 Steam Explosion (Autohydrolysis)

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials. In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160–260 ^{o}C for several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. The factors that affect steam explosion pretreatment are residence time, temperature, chip size and moisture content. Addition of H_2SO_4 or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicellulose. Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin–carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in downstream processes. Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed by water to remove the inhibitory materials along with water-soluble hemicelluloses. The water wash decreases the overall